Original Article

Evaluation of petroleum ether heartwood extract of *Cedrus deodara* in healthy and diabetic rats

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Abstract

Background and Aim: Global prevalence of diabetes is increasing rapidly. Herbal medicinal plants for diabetes give good results with fewer side effects. The current study was conducted in a *Cedrus deodara* plant to evaluate the hypoglycemic activity and antidiabetic activity of petroleum ether extract of *Cedrus deodara* (PEECD) which may be helpful for diabetes patient.

Methods: PEECD at three different doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg was administered to normal rats (n = 36, group = 6) and alloxan-induced diabetic rats (n = 36, group = 6). Glucose tolerance test (GTT) was also assessed in experimental models. Statistical analysis used was one-way ANOVAs.

Results: With the 400 mg/kg dose, there was a definite hypoglycemic response which was markedly significant and comparable to standard drug glibenclamide. Both the PEECD 200 and 400 mg/kg dose significantly reduced blood glucose level of diabetic rats nearer to the normal level by 21st day. The PEECD 400 mg/kg dose showed significant effectiveness in reducing the GTT values which was comparable to standard.

Conclusion: These test results justified to some extent that the folklore medicine and PEECD could be added to traditional preparations for the ailment of various diabetes-associated complications by further study.

Key words: Antidiabetic activity, *Cedrus deodara*, diabetes

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INTRODUCTION

Diabetes is a metabolic disease characterized by hyperglycemia resulting from disturbance in insulin secretion and insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[1] Prevalence of diabetes is increasing with lifestyle changes leading to sedentary habit. Global prevalence of diabetes in the year 2000 was estimated to be around 171 million, and this global burden of diabetes mellitus is expected to almost double by the year 2030.[2] Most of the antidiabetic agent (except metformin) exhibits adverse side effects particularly increasing the incidence of cardiovascular adverse events.[3] Therefore, there is a need to explore alternative therapeutic strategies to manage diabetes with fewer side effects. The WHO Expert Committee have also recommended that the traditional plant treatment for diabetes needs to be further explored.[4] Marles and Farnsworth reviewed the role of more than 1200 species of plants having antidiabetic activity.[5] Based on the review of herbal medicinal plants that are effective in diabetes, we have selected a *Cedrus deodara* plant for the present study. *C. deodara*

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belong to the family of *Pinaceae* (English - Himalayan cedar, Hindi - Deodar, Sanskrit/Bengali - Devadaru) is an evergreen tree, growing extensively in the Mediterranean region and the Western Himalayas.[6] Gupta *et al*., 2011, have reviewed the therapeutic potential of *C. deodara* plant as an antidiabetic agent along with several other effects such as antimicrobial, cytotoxic, antispasmodic, anti-inflammatory, analgesic, immunomodulatory, antioxidant, and anxiolytic activity.[7] Studies exploring antidiabetic potential of *C. deodara* are very limited. The present study is proposed to investigate the hypoglycemic activity and antidiabetic activity of petroleum ether extract of *Cedrus deodara* (PEECD).

MATERIALS AND METHODS

Drugs and chemicals

Glibenclamide tablet (Aventis Pharma Ltd., Mumbai, India), Tween-80 (Rankem, Ranbaxy Fine Chemicals Ltd., New Delhi, India). All the other solvents and chemicals used for extraction and phytochemical investigation were purchased from S.D Fine Chemicals Pvt. Ltd., Mumbai.

Collection of plant material

Heartwood of *C. deodara* was collected from Sugandha Kesari Depot, Mamulpet, Bengaluru, Karnataka, which was authenticated by the Department of Botany, GKVK University, Bengaluru, Karnataka.

Plant extraction

Eighty grams air-dried powdered plant materials were successively extracted using Soxhlet apparatus with 60–80% petroleum ether for 72 h. The extract was then concentrated by distilling the solvent and evaporating them to dryness at low temperature. Final traces of solvent were removed under pressure using rotary vacuum flask evaporator, and the extract was preserved for the study.

Phytochemical investigation

The PEECD has been subjected to various qualitative tests for the identification of various chemical constituents.[8-10]

Experimental animals

Adult male Albino Wistar rats (150–200 g) were procured from Bioneeds, Nelamangala, Tumkur (and then). The animals were acclimatized at the animal house of PES Institute of Medical Science and Research, Kuppam (Andhra Pradesh) for 7 days under standard husbandry conditions, i.e., room temperature of 25°C ± 10°C, relative humidity 45–55%, and a 12:12 h light/dark cycle. They were fed with standard rat pellet (Pranav Agro Industries Ltd., Bengaluru, India) and water *ad libitum*. The approval of the Institutional Animal Ethical Committee (Andhra Pradesh) was taken before the experiments, and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Acute toxicity studies

Acute oral toxicity test was carried out according to Organisation for Economic Co-operation and Development guideline no. 425 (up and down procedure). PEECD was safe up to 500 mg/kg and was lethal at 2000 mg/kg dose. The lethal dose 50 of PEECD was 1098 mg/kg.[11]

Collection of serum samples

The blood was drawn from the retro-orbital plexus of the rats under light ether anesthesia on 0, 7th, 14th, and 21st day. The blood samples were allowed to clot for 30 min at room temperature, and the serum samples were collected immediately for the biochemical analysis.

Biochemical analysis

Serum glucose level (SGL) was estimated colorimetrically by glucose-oxidase-peroxidase (GOD/POD) method using Glucose Estimation Kit (Span Diagnostic Ltd., Surat, India).

Preparation of test sample

The Petroleum ether heartwood extract of *C. deodara* was suspended in water in the presence of 3% v/v Tween-80 solution. Each time fresh preparations of the extracts were prepared as when required.

Preparation of glibenclamide solution

Glibenclamide tablet of 5 mg was dissolved in 10 ml of distilled water to give 0.5 mg/ml solution. This solution was administered at a dose of 5 mg/kg body weight (b.w).

Assessment of hypoglycemic activity

In this experiment, a total of 36 adult healthy male rats were used and they were randomly divided into six groups of six animals each. All the samples were administered through oral route in the present study. Group 1 received 10 ml/kg of distilled water (normal control), Group 2 received 2 ml of 3% v/v Tween-80 in water (vehicle control), Group 3 received 5 mg/kg of glibenclamide (standard), Group 4 received 100 mg/kg of PEECD (Test 1), Group 5 received 200 mg/kg of PEECD (Test 2), Group 6 received 400 mg/kg of PEECD (Test 3). The blood samples were collected from overnight fasted rats from retro orbital plexus just before the treatment and also at an interval of 1 h after the administration. According to the procedure, SGLs were estimated colorimetrically using GOD/POD method. The administration of drugs was continued for next 21 days, and SGLs were estimated on 0, 7th, 14th, and 21st days.

Induction of diabetes

Diabetes was induced in 16 h fasted rats by single intraperitoneal injection of 125 mg/kg of freshly prepared alloxan solution in normal saline. After alloxan injection, 10% w/v glucose solution was given in feeding bottles for next 24 h to prevent hypoglycemia. After 7 days, rats with marked hyperglycemia (fasting SGL >200 mg/dl) were selected for the study. Animals (no. 6) belonging to Group 1 were not treated with alloxan.

Assessment of antidiabetic activity

A total of 36 rats (6 control rats and 30 diabetic rats) were used. Diabetic rats were divided into five groups of six animals each. Group 1 (nondiabetic healthy rats) received 10 ml/kg of distilled water (Normal Control), Group 2 received 2 ml of 3% v/v Tween-80 in water (vehicle control), Group 3 received 5 mg/kg of glibenclamide (standard), and Group 4, 5, and 6 received 100 mg/kg, 200 mg/kg, and 400 mg/kg of PEECD, respectively. The collection of blood samples and estimation of SGLs were done by same methods as in the assessment of hypoglycemic activity.

Oral glucose tolerance test in alloxan-induced diabetic rats

On 22nd day, the oral glucose tolerance test (GTT) was performed on all the animals of alloxan-induced diabetic groups. After 1 h of respective samples, the rats were orally administered (treated) with 2 g/kg of glucose. The blood samples were collected at "0" h, i.e. just before the administration of glucose load and at 1st, 2nd, and 3rd h after the administration of glucose load. SGLs were estimated.

Statistical analysis of data

The statistical analysis was done using the software SPSS Statistics Developer 21.0.0 (IBM, Chicago, USA). The values were expressed as a mean ± standard deviation. The effect was compared using one-way ANOVA followed by Tukey's test. $P < 0.05$ was considered as statistically significant.

RESULTS

Hypoglycemic activity of PPECD

Glibenclamide produced a fall in SGLs in healthy rats and reached the hypoglycemic level on the 21st day [Table 1]. PEECD 100 mg/kg dose, the fall in SGLs is less as compared to glibenclamide on 7th and 14th day, but on the 21st day, the fall was significant. With PEECD 200 mg/kg dose, the fall in SGL was more marked. The 400 mg/kg dose had a definite hypoglycemic response and was comparable to glibenclamide.

Antidiabetic activity of PPECD

The normal control and vehicle-treated group did not show any variation on SGL in alloxan-induced diabetic rats. Glibenclamide produced a reduction in SGL on the 7th day, 14th day, and on the 21st day, which is nearer to the normal SGL (in alloxan-induced rats). The PEECD 100 mg/kg and 200 mg/kg produced a fall in SGL on the 7th, 14th, and 21st days [Table 2]. The PEECD 400 mg/kg produced a fall in SGLs on the 7th, 14th, and 21st days which is nearer to the normal level. The PEECD 200 and 400 mg/kg significantly reduced SGLs of diabetic rats, nearer to the normal level by the 21st day.

Glucose tolerance test on alloxan-induced diabetic rats

Table 3 shows GTT in alloxan-induced diabetic rats after 21st day of drug treatment. The initial SGL values were high in vehicle-treated group of diabetic rats, whereas lower and not uniform in drug-treated groups, which have been receiving drugs for the past 21st days. There was a rise in SGL at 1st h, and it started falling at 2nd h and 3rd h and reached the initial level at 3rd h. In vehicle-treated group, the initial SGLs were very high (diabetic) and at 1st h, there was a significant rise and continued to be high even after 3rd h. In glibenclamide-treated group, there was a slight rise SGL at 1st h and reached to near the normal level at 3rd h. In PEECD 100 mg treated group, SGL started rising at the 1st, 2nd, and at the 3rd h, the level was slightly reduced but above the 1st h level. In PEECD 200 mg treated group, SGL is increased at 1st h and decreased at 2nd h and 3rd h. At 3rd h levels were higher than the initial value which is significant. PEECD 100 mg/kg and 200 mg/kg in the treated group, the SGLs have not reached initial values at the end of the GTT. PEECD 400 mg/kg treated group has shown a slight rise in SGL at 1 h which came back to normal at initial level by 3rd h. The PEECD 400 mg/kg showed significant effectiveness in reducing the GTT values which is comparable to glibenclamide.

DISCUSSION

In the present study, PPECD was evaluated for its effectiveness in reducing SGL concentration and was compared with conventional oral antihyperglycemic drug glibenclamide. Glibenclamide is an oral antidiabetic preparation and very frequently used as a reference drug in antidiabetic activity.[12,13] The PEECD was evaluated as antidiabetic agent and found to have SGL lowering effects in normoglycemic as well as hyperglycemic rats. The PEECD at all doses has the ability to produce hypoglycemic effect in fasting rats. The degree of SGL reduction produced by the extract at 400 mg/kg b.w dose was comparable to glibenclamide-treated group which was used as standard drug. This

Table 1: Effect of drugs on blood glucose levels in normal rats

Treatment group	Dose	Blood glucose level (mg/dl)			
		0 day	7th day	14th day	21th day
Distilled water (control)	10ml/kg	86.19±1.91	87.98±1.91	86.09±0.69	87.49±1.3
Vehicle control	2 ml	92.36±1.77#	93.81±2.02#	90.21±3.09#	95.49±2.04#
Glibenclamide (standard)	5 mg/kg	90.21±2.66#	72.73±2.10#	59.66±2.63#	40.42±2.95#
PEECD	100 mg/kg	92.01±1.96#	88.95±1.28*	79.77±2.03#*	70.95±2.5#*
PEECD	200 mg/kg	96.09±0.87#*	86.25±1.02*	74.31±1.93#*	66.31±0.7#*
PEECD	400 mg/kg	95.24±1.18#*	77.78±1.23#*	68.5±1.28#*	55.41±1.84#*

Values represented as mean±SD (n=6). Significant differences (P<0.05) as compared to standard have been superscripted with * and significant differences (P<0.05) as compared to control have been superscripted with #. PEECD: Petroleum ether extract of cedrus deodara

Table 2: Effect of drugs on blood glucose levels in alloxan induced rats

Treatment group	Dose	Blood glucose level (mg/dl)			
		0 day	7th day	14th day	21th day
Distilled water (control)	10ml/kg	84.5±3.06	88.16±2.82	86.33±1.81	87.5±1.6
Vehicle control	2 ml	252.16±8.53#	244.33±9.43#	246.5±5.61#	247.33±5.92#
Glibenclamide (standard)	5 mg/kg	292.83±10.24#	146.66±3.92#	126±3.03#	104.5±2.39#
PEECD	100 mg/kg	290.95±9.95#	235.3±13.6#*	210.57±9.22#*	192.81±5.32#*
PEECD	200 mg/kg	281.5±8.85#	233.33±6.95#*	174.16±2.73#*	128.16±4.31#*
PEECD	400 mg/kg	270.33±6.65#*	231.5±6.35#*	152.5±6.09#*	122.83±4.67#*

Values represented as mean±SD (P=6). Significant differences (P<0.05) as compared to standard have been superscripted with * and significant differences (P<0.05) as compared to control have been superscripted with #. PEECD: Petroleum ether extract of cedrus deodara

Table 3: Effect of drugs on glucose tolerance test (GTT) in alloxan induced diabetic rats on 22nd day of experimental

Treatment group	Dose	blood glucose level (mg/dl)			
		0 hr	1st hr	2nd hr	3rd hr
Distilled water (control)	10ml/kg	90.21±7.49	140.12±6.54	103.97±9.21	96.41±5.5
Vehicle control	2 ml	299±14.95#	447.24±10.22#	342.09±6.73#	307.45±13.38#
Glibenclamide (standard)	5 mg/kg	87.8±2.82#	103.78±4.11#	95.28±4.9#	93.25±9.38#
PEECD	100 mg/kg	136.4±2.56#*	145.8±4.8*	179.8±7.33#*	160.5±6.50#*
PEECD	200 mg/kg	135.1±8.92#*	155.59±11.77#*	141.51±9.29#*	140.23±6.3#*
PEECD	400 mg/kg	122.36±5.93#*	134.64±5.53#*	127.25±6.63#*	125.25±4.69#*

Values represented as mean±S.D (n=6). Significant differences (P<0.05) as compared to standard have been superscripted with * and significant differences (P<0.05) as compared to control have been superscripted with #. PEECD: Petroleum ether extract of cedrus deodara

hypoglycemic activity of PEECD could be due to the presence of several constituents such as alkaloids, flavonoids phenol. An earlier phytochemical study on PEECD has reported the presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins, tannins, and proteins. Alkaloids, flavonoids, and phenolic acids are known to be bioactive antidiabetic substances.[14] In the antihyperglycemic evaluation, the PEECD at 200 mg/kg b.w and 400 mg/kg b.w doses could produce the reduction in SGL comparable to standard drug glibenclamide on 14th and 21st days in alloxan-induced diabetic rats. This observation indicates that PEECD at 200 mg/kg b.w and 400 mg/kg b.w doses has good antihyperglycemic action. Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is a toxic glucose analog which selectively destroys insulin-producing β-pancreatic cells when administered to rodents and many other animal species. This causes insulin dependent diabetes mellitus (alloxan diabetes) in these animals with characteristics similar to type-1 diabetes in human.[15] An earlier studies have also reported that alloxan has a destructive effect on the β-cells of the pancreas.[16,17] This antihyperglycemic action of PEECD in alloxan-induced diabetic rats may be due to the presence of constituents such as flavonoids and alkaloids which decrease the fasting SGL and also postprandial SGL by inhibiting alpha-glucosidase activity.[18] The antihyperglycemic action of PEECD may be due to flavonoids-mediated regeneration of damaged β-cells in the alloxan-induced diabetic rats.[19] In addition, PEECD may have glycolytic and glycogenic processes with accompanying decrease in glycogenolysis and gluconeogenesis.[20] GTT was conducted on 22nd day of experimentation. The results showed that PEECD at 400 mg/kg b.w dose is effective in controlling the postprandial hyperglycemia comparable to glibenclamide. Earlier studies have also reported that *C. deodara* has hypoglycemic and antihyperglycemic activity using different drug induced in the different animal models.[21] Our observations suggest that PEECD mediates hypoglycemic, antihyperglycemic, and postprandial antihyperglycemic activity.

Limitations of the study

We have not assessed the details of chemical composition of the extract and their effects on the parameters studied.

CONCLUSION

The present study showed that PEECD has the ability to reduce the SGLs. This result provides the basis and justifying to some extent the use of the plant in folklore medicine. The current study did not explore the mechanism of action of the individual constituents of PEECD; nevertheless, it confirms the initial evidence and supports the fact that it possess potential antidiabetic properties. Further randomized controlled trials are warranted to confirm its effectiveness in human subjects.

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Conflicts of interest

There are no conflicts of interest.

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